

Gastrointestinal Bacteria of Certain Antarctic Birds and Mammals

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An investigation was carried out of the aerobic gram-negative intestinal flora of 158 penguins, 30 skuas, and 66 seals from the Antarctic and sub-Antarctic zones and Phillip Island. Among penguins and seals, 17% and 11%, respectively, were devoid of aerobic enteric organisms, but microscopic examination showed the presence of other bacteria. The coliforms outnumbered the paracolons and nonlactose-fermenting bacteria; irregular coliform types and *Escherichia coli* were predominant. The present study indicated the effect of diet on the composition of the intestinal flora of the birds and mammals examined.

Bunt (2) found the feces of some antarctic birds to be sterile and some devoid of coliform bacteria. On the basis of his and other observations on birds and mammals, he considered the lack of bacterial growth in the gut to be due possibly to competition for nutrients or the production of antibiotic substances by some members of the intestinal flora. However, he did not accept as reasonable a previously made suggestion of a sterile or almost sterile diet to be responsible for the absence of bacteria. Sieburth (7) did not find "bacteriologically sterile" antarctic birds, but he recorded the absence of aerobes and the presence of obligate anaerobes in some intestinal samples.

The present investigation was concerned mostly with the study of aerobic gram-negative gastrointestinal bacteria in penguins, skuas, and seals from various localities during the period of 1962 to 1965. The aim was to determine the types of organisms present and to investigate the possibility of different feeding habits exerting an influence on the composition of the microflora.

The main part of this paper deals with penguins, as within this one family data could be collected on the influence of different environmental, climatological, and physiological conditions. These birds are distributed over a wide territory in the southern hemisphere, they have an interesting ecology, partly maritime and partly terrestrial, and they are able to ingest seawater or fresh water.

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MATERIALS AND METHODS

Animals. The samples were collected from representatives of two families of oceanic birds, namely the penguins and the skuas, and from one family of mammals, the seals.

The material came from animals in the localities shown in Fig. 1: Antarctic (Mawson, Wilkes, Cape Adare, and Cape Hallett on the Continent; Franklin, Coulman, Beaufort, and Possession Islands in the Ross Sea; and Balleny Islands group), sub-Antarctic (Macquarie Island), and Phillip Island in the southern part of Australia. The area ranged in latitude from 38°44'S to 76°07'S. The birds and mammals examined in various localities are listed in Table 1. In all, eight species of penguins, two of skuas, and five of seals were under study.

Specimens. The collection of specimens was carried out mostly in the field, in the summer months from October to March. In the examination of each animal, a swab was taken from the ventriculus, the middle intestine, the cloaca of birds, and the rectum of mammals and was immersed in Stuart's (9) preservative medium. Frequently, an additional swab was taken from each segment and placed in cooked-meat medium. The specimens were processed at either the Australian National Antarctic Research Expeditions' Station at Wilkes, Antarctica, or at the Antarctic Division Laboratory in Melbourne. Working conditions in both laboratories were excellent.

Media. Dehydrated culture media (Difco) were used. The swabs were plated out on MacConkey and deoxycholate agar and then immersed in sodium selenite enrichment medium for subsequent subculture to SS agar. Selected colonies were seeded into peptone water for further examination in differential media, and the peptone water culture was further used for motility and indole tests. The cultures were in-

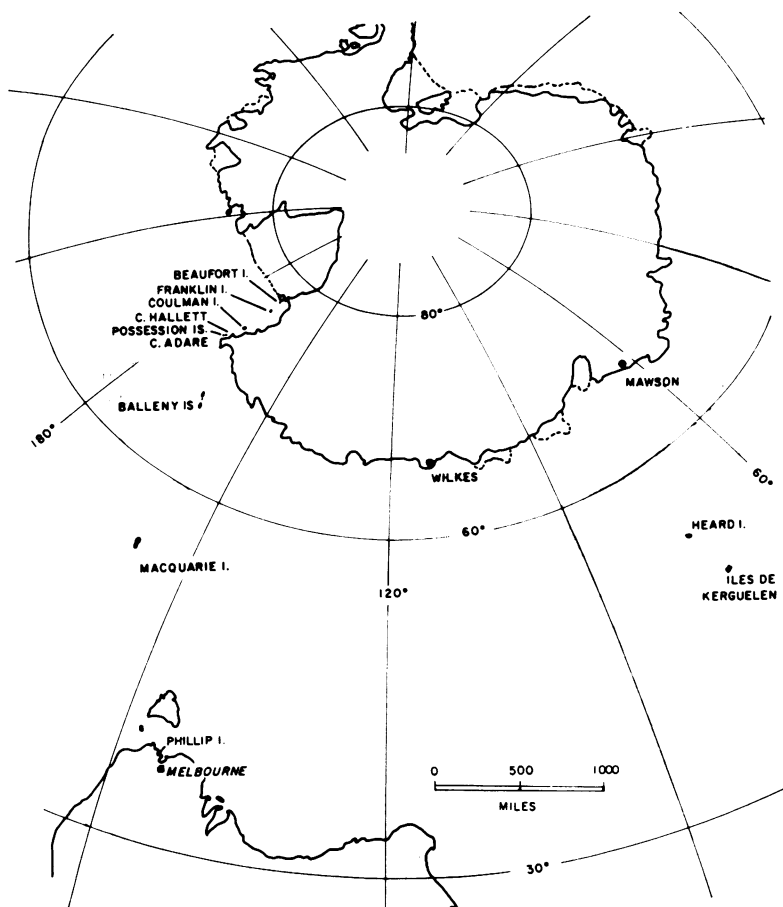


FIG. 1. Map of Antarctic, sub-Antarctic, and Phillip Island.

TABLE 1. Families and species of birds and mammals examined in various localities

Family	Species	Locality
Penguins (<i>Spheniscidae</i>)	Adélie, Emperor, and Chinstrap King, Royal, Rockhopper, and Gentoo Fairy	Antarctic Sub-Antarctic Phillip Island (temperate zone)
Skuas (<i>Stercorariidae</i>)	McCormick Brown	Antarctic Sub-Antarctic
Seals (<i>Phocidae</i>)	Leopard, Crabeater, Weddell, and Ross Elephant	Antarctic Sub-Antarctic

cubated aerobically at 37 C. The diagram on the use of media is shown in Fig. 2.

Identification of bacteria. Gram-stained smears from the swabs were examined microscopically. The bacteria were identified according to *Bergey's Manual* (7th ed.); however, within the *Enterobacteriaceae* the more recently accepted nomenclature was introduced, thus *Escherichia freundii* was replaced by *Citrobacter freundii* and *Aerobacter* by *Enterobacter* (3). The

Paracolobactrum (paracolon) genus, denoting the late lactose-fermenting coliform bacteria, was retained for convenience although taxonomically this group is not considered to be valid (4).

Coliform bacteria were divided into genera and species (*Bergey's Manual*, 7th ed.), and "irregular" types (10) on the basis of IMViC reactions, which include indole (I), methyl red (M), Voges-Proskauer (V), and citrate (C) tests, and also on motility and

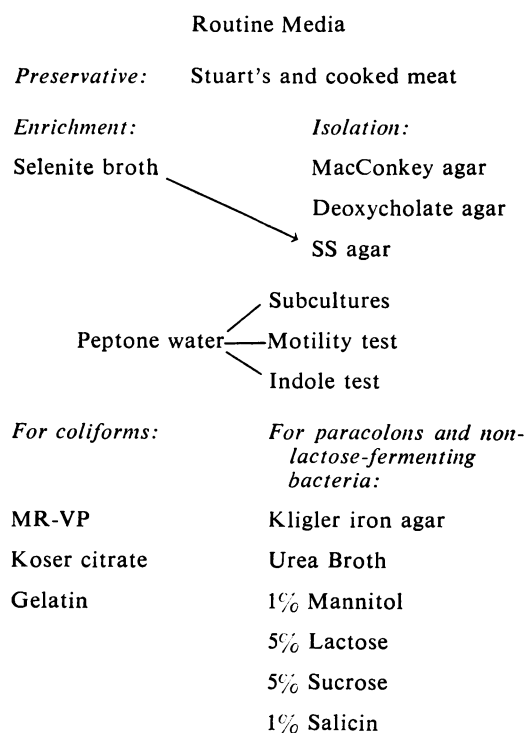


FIG. 2. Diagram of the use of media for cultures of bacteria. Abbreviations: MR, methyl red; VP, Voges-Proskauer.

gelatin liquefaction tests. The I test was performed with a xylol extract and Ehrlich reagent. The M test was carried out after incubation for 5 days at 30 C, and the V test after 2 days at 30 C, by using creatine and sodium hydroxide according to O'Meara's modification. C utilization was recorded after 48 hr of incubation. The nonlactose-fermenting colonies were biochemically characterized by reactions in Kligler's lactose-glucose ferrous sulfate agar, urea broth, and mannitol peptone water. Differentiation of *Paracolo-bactrum* species, resembling *Salmonella*, was carried out on the basis of indole test and fermentation of 5% lactose, 5% sucrose, and 1% salicin.

Apart from the routine investigations of gram-negative enteric bacteria, microscopic examination of the specimens was carried out. Observations which indicated the presence of gram-positive organisms were followed by cultivation on blood agar and tellurite agar media.

RESULTS

The incidence, the frequency of isolation, and the identification of aerobic gram-negative bacteria encountered in the gut of penguins, skuas, and seals are presented in Tables 2, 3, and 4. Some observations were too detailed for inclusion in the tables, and the relevant data are presented in the text.

Penguins. Of 158 birds examined, of which the majority were from the Antarctic and sub-Antarctic region and 11 from Phillip Island, 27 (17%) did not harbor in their intestine any aerobic gram-negative bacteria. In the total number of 786 specimens, 55% were found to be positive, and the coliforms outnumbered the paracolons and nonlactose-fermenting bacteria in a ratio of 13:1. The isolates came from various segments of the gut. The highest frequency of isolations was usually from cloacal swabs; this was especially evident in Gentoo and Royal penguins from the sub-Antarctic zone. However, in some Adélie penguins and all Emperor penguins from the Antarctic region a larger number of strains came from the middle intestine. The microflora of penguins from Phillip Island was more evenly distributed in the gut.

The coliforms were the predominant isolates from penguins, especially the irregular types and *Escherichia coli*. Among nonlactose-fermenting bacteria, *Alcaligenes faecalis* was most frequently detected, and paracolons were next in incidence.

Additional data on the microflora of penguins are presented in Table 5. Among the birds from the Antarctic, sub-Antarctic, and Phillip Island, a marked difference was noted in the incidence of specimens devoid of gram-negative bacteria, the figures being 21, 10, and 4%, respectively. Further, a difference was evident in the frequency of isolations of *E. coli* strains, the highest percentage coming from specimens from Phillip Island.

Table 6 shows the distribution of bacterial strains in the gut in various species of penguins. The Adélie species in the Antarctic zone was represented by the largest number of birds and, in proportion, the largest number of irregular coliform types and *A. faecalis* strains came from this source. *Pseudomonas* was detected in material from Gentoo penguins.

Skuas. Among 40 of these birds, none was devoid of aerobic gram-negative microflora; of 236 specimens examined, 65% were positive. The ratio of coliforms to paracolons and nonlactose-fermenting bacteria was 13:1, similar to that in penguins. The distribution of bacterial strains in the intestinal segments was more uniform than in the penguins, and *E. coli* was predominant. *A. faecalis* and paracolons appeared in both species of skuas, and *Proteus vulgaris* appeared in McCormick skuas.

Seals. Of the 66 mammals examined, aerobic gram-negative bacteria were undetected in seven (11%). Of 300 samples processed, 57% were found to be positive. The ratio of coliforms to paracolons and nonlactose-fermenting bacteria was 8:1, lower than in the birds. The highest frequency of isolations was recorded from the rectum

TABLE 2. Incidence and frequency of isolation (aerobically at 37 C) of gram-negative bacteria from the gut of animals

Type and no. of animal	Animals with positive specimens		Specimens			No. of isolations			
	No.	Per cent	Total no.	Positive		Total	Coliforms (I)	Paracolons and nonlactose-fermenters (II)	Ratio of groups I to II
				No.	Per cent				
Penguins (158)	131	83	786	429	55	916	852	64	13:1
Skuas (40)	40	100	236	154	65	353	327	26	13:1
Seals (66)	59	89	300	170	57	316	280	36	8:1

TABLE 3. Frequency of isolation (aerobically at 37 C) of gram-negative bacteria from gastrointestinal segments of animals

Type and no. of animal	Organ	Specimens			No. of isolations			Ratio of groups (I to II)
		Total no.	Positive		Total no.	Coliforms (I)	Paracolons and non-lactose-fermenters (II)	
			No.	Per cent				
Penguins (158)	Ventriculus	258	129	50	255	236	19	12:1
	Middle intestine	262	136	52	288	264	24	11:1
	Cloaca	266	164	62	373	352	21	17:1
Skuas (40)	Ventriculus	78	48	62	110	103	7	15:1
	Middle intestine	79	51	65	104	94	10	9:1
	Cloaca	79	55	70	139	130	9	14:1
Seals (66)	Ventriculus	100	46	46	78	64	14	5:1
	Middle intestine	100	50	50	97	84	13	6:1
	Rectum	100	74	74	141	132	9	15:1

TABLE 4. Frequency of isolation (aerobically at 37 C) and identification of gram-negative bacteria from the gut of animals

Type and no. of animal	Total no. of isolations	No. of isolations of bacterial strains							
		Coliforms				Paracolons and nonlactose-fermenters			
		<i>E. coli</i>	<i>C. freundii</i>	<i>Enterobacter</i>	Irregular	Paracolon	<i>Alcaligenes faecalis</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas</i>
Penguins (158)	916	386	9	8	449	17	45	0	2
Skuas (40)	353	204	10	2	111	10	13	3	0
Seals (66)	316	130	8	2	140	29	6	1	0

specimens and these yielded the highest number of *E. coli* strains, whereas the irregular types were comparatively evenly distributed in the gut. Paracolons were more frequently encountered in the higher sections of the gut than in the rectum. These strains and *A. faecalis* came from seals in the Antarctic and sub-Antarctic region, whereas *P. vulgaris* was isolated from an Elephant seal.

Microscopic examinations indicated that all

specimens contained microorganisms. The isolates of gram-positive bacteria included *Streptococcus faecalis* and diphtheroids from penguins and *Staphylococcus epidermidis* from the three families of animals under investigation.

DISCUSSION

The present study indicated that the microflora of the gut of animals may depend on their loca-

TABLE 5. Incidence of "negative" specimens (devoid of aerobic gram-negative bacteria) and frequency of isolation of *E. coli* from penguins in various locations

Location	No. of penguins examined	Penguins with "negative" specimens		No. of isolations		
		No.	Per cent	Coliforms	<i>E. coli</i>	
					No.	Per cent
Antarctic	106	22	21	363	62	17
Sub-Antarctic	41	4	10	391	208	53
Phillip Island	11	1	4	162	116	73
Phillip Island ^a	15	0	1	42	32	148

^a These specimens were collected in the course of a previous investigation.

TABLE 6. Frequency of isolation (aerobically at 37 C) of gram-negative bacteria from the gut of penguins in various locations

Location	Species and no. of penguins	No. of sterile birds	Total no. of isolations	No. of isolations of bacterial strains							
				Coliforms				Paracolons and nonlactose-fermenters			
				<i>E. coli</i>	<i>C. freundii</i>	<i>Enterobacter</i>	Irregular	Paracolon	<i>Alcaligenes faecalis</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas</i>
Antarctic	Adélie (95)	19	335	46	7	7	220	12	43	0	0
	Emperor (8)	2	21	14	0	0	7	0	0	0	0
	Chinstrap (3)	1	7	2	0	0	5	0	0	0	0
Sub-Antarctic	Rockhopper (10)	0	114	53	0	1	59	0	1	0	0
	King (8)	2	144	75	2	0	67	0	0	0	0
	Gentoo (10)	2	45	25	0	0	13	4	1	0	2
Phillip Island	Royal (13)	0	88	55	0	0	33	0	0	0	0
	Fairy (11)	1	162	116	0	0	45	1	0	0	0

tion and, consequently, on the available diet. Among the penguins and seals, which feed on crustaceae, cephalopods, and fish, the incidence of animals devoid of gram-negative flora was 17 and 11%, respectively; however, in skuas, which have predatory habits, the incidence was nil. The predominance of coliforms to paracolons and nonlactose-fermenting bacteria was evident and usually most pronounced in the caecum or rectum.

In the coliform group, a high frequency of isolations of *E. coli* and irregular types was recorded. In the late and nonlactose-fermenting group, either paracolons or *A. faecalis* was predominant, whereas *P. vulgaris* and *Pseudomonas* strains were only occasionally encountered.

The penguins presented a suitable subject to illustrate the influence of diet on intestinal flora. Among penguins from the Antarctic zone, birds which feed on crustaceae and fish, 21% were devoid of aerobic gram-negative bacteria. In the

sub-Antarctic region, where the penguins feed on crustaceae, cephalopoda, and fish, the incidence of birds with "negative" specimens was 10%, whereas the Phillip Island penguins, which exist mainly on a diet of small fish, showed the lowest figure of 4%. Of the Antarctic birds examined, 95 belonged to Adélie species; specimens from 19 of them gave the highest incidence, namely 20%, of "negative specimens." The stomachs of 15 of these birds were full of euphasids at different stages of digestion, and the remaining four birds had empty stomachs. In many of the full stomachs, the green algae *Phaeocystis pouchetti* was found. It may be noted that sea water in the Antarctic zone is reported to be richer in plankton than the Phillip Island region.

Sieburth (7) challenged the validity of reports of "bacteriologically sterile" birds by demonstrating in "negative" specimens the presence of anaerobic bacteria, whereas conclusions of other writers were based on observations of absence of

growth under aerobic conditions. In an extensive survey of mutton birds, Mushin and Ashburner (6) also reported the absence of aerobic enteric bacteria in some birds, but anaerobic cultivation or microscopic examination indicated the presence of other types of organisms. The quantitative discrepancy between microscopically positive and culturally negative specimens suggested the presence of antibacterial substances in the diet of these birds. The antibacterial activity of green algae, *Phaeocystis*, similar to *P. pouchetti*, was shown by Sieburth (8) to be due to acrylic acid as the antibiotic principle. The algal substance was identified in the stomach contents of euphasids, which are the diet of penguins. McBee (5) also reported the antibiotic effect of algae in the digestive tract of shrimps, which form a large part of the diet of Antarctic fish.

In the penguins from three locations, a marked difference was noted in the type of coliform flora, thus the frequency of isolation of *E. coli* from the birds in the Antarctic zone was 17%; the figure rose to 53% in specimens from the sub-Antarctic and reached 73% in material from Phillip Island.

Similarly, in the examination of samples from seals a correlation was found between the intestinal flora and the diet of these mammals. The Crabeaters which feed mainly on crustaceae had only 7% of positive specimens as against 87% from Leopard seals which have predatory habits.

The observations made at Wilkes on specimens from the gut of 70 Adélie penguins indicated the influence of environmental conditions. Samples collected in October, when the penguins begin to live terrestrially after having spent the winter months in pack-ice, yielded very poor growth, although the growth became more abundant as the period of terrestrial living extended into the months of March and April. Further, there was a definite trend in the appearance of bacterial types. The incidence of paracolons and nonlactose-fermenting bacteria was 3% in specimens obtained between October and December, whereas a rise to 29% was shown in the material collected in

March and April. The changes in microflora seemed to be the effect of diet.

There is no evidence of influence of climatological conditions on the presence and composition of intestinal flora in the Antarctic animals. Biotelemetry studies (1) on penguins showed fluctuations in temperature in various organs such as proventriculus and cloaca, the temperature ranging from 37.5 C to 40.0 C, the average being 38.9 C. The birds and mammals examined in the present study were warm-blooded animals, anatomically and physiologically equipped to retain the average body temperature under different weather conditions.

Thus, the present investigation indicated the influence of diet habits on the gastrointestinal flora of the examined animals.

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